Editorial Response: Predicting Protection Against Encapsulated Pathogens

Patients with selective immune defects have provided vivid evidence of the specific host factors that mediate defense against the pneumococcus. Rates of invasive pneumococcal infections may be increased among patients with deficiencies of complement components (involving either the classical or alternative pathway) [1–3], decreased phagocyte number and function (including neutropenia, asplenia, and perhaps specific Fcγ receptor IIa allotypes) [3–7], impaired antibody production (e.g., hypogammaglobulinemia, IgG2 subclass deficiency, and selective unresponsiveness to polysaccharides) [8–10], or combined defects (e.g., sickle cell disease, asplenia, and HIV-1 infection) [11–13]. Of these factors, only levels of capsule-specific antibody are currently amenable to modulation by medical intervention.

See article by Romero-Steiner et al. on pages 281–8.

Augmenting levels of specific antibody by immunization with epidemiologically and chemically defined capsular polysaccharides has provided the focus for prevention of serious pneumococcal infections in patients at increased risk. Early descriptions of the successful prevention of these infections with polysaccharide vaccines [14–16] in high-risk but otherwise healthy young adults confirmed the biological validity of this strategy. However, since these initial successes, the efficacy of the vaccine has been tested and contentiously debated in the largest group of susceptible adults, the elderly.

In aggregate, evidence to date suggests that the multivalent pneumococcal capsular polysaccharide vaccine affords protection (50%–75%) against invasive disease (e.g., bacteremia and meningitis) [17–19] rather than pneumonia in older adults [20, 21]. Because of the expense and logistical challenges of performing prospective, randomized clinical trials in the elderly, investigators have sought to define correlates of protection against pneumococcal disease in order to evaluate the many new and innovative protein-associated polysaccharide vaccine candidates in development. Conspicuous in this approach has been the measurement of capsule-specific antibodies by ELISA.

Recent efforts have been directed toward improving the specificity and standardizing the quantitation of capsule-specific antibodies by ELISA [22, 23], as well as defining the "functional activity" of these antibodies [24]. In this issue, Romero-Steiner et al. report that levels of capsule-specific IgG after pneumococcal vaccination were significantly lower (for two of five serotypes studied) in 46 elderly subjects than in 12 young adults [25]. More important, functional immune responses assessed on the basis of in vitro killing activity were significantly reduced for all serotypes in the elderly, compared with those in young subjects.

Functional immune responses measured in this assay were not highly correlated with antibody levels determined by ELISA but did correlate with relative antibody avidities measured. Thus, the findings of Romero-Steiner et al. support the importance of determining functional measures of immune response to pneumococcal vaccination in the elderly and suggest that analysis of specific antibody avidity may be a reliable surrogate functional measure to employ in future studies of vaccine response.

"Avidity" has been defined as the "functional affinity" of antibodies. Avidity is determined by at least two parameters [26]. The first is the true or intrinsic affinity, the attractive force between antigen- and antibody-binding sites. The second is the number of antigen-binding sites on the antibody, e.g., 2 sites for IgG, 10–12 for IgM, and 2–4 or more for IgA. The affinity and/or avidity of specific antibodies has been correlated with their functional activity in vitro against many pathogens [27], including other bacteria with polysaccharide capsules, such as *Haemophilus influenzae* type b [28, 29] and *Neisseria meningitidis* [30]. Romero-Steiner et al. now report that the avidity of IgG for *Streptococcus pneumoniae* capsular polysaccharides correlates with the ability of sera to mediate complement-dependent killing of the organism by phagocytes in a standardized in vitro assay.

These investigators measured the avidity of IgG to capsular polysaccharides of *S. pneumoniae* by inhibition of antigen binding to the solid-phase ELISA antigen with increasing concentrations of ammonium thiocyanate. This chaotropic agent dissociates antigen-antibody complexes [31]. Among the other available methods for determining affinity or avidity of specific antibodies (e.g., equilibrium dialysis, competitive inhibition ELISA, and surface plasmon resonance) [32–36], thiocyanate elution is among the easiest and most inexpensive to perform. Such ease of performance provides access to and standardization of the assay in large numbers of laboratories worldwide. On the basis of the experience and success with *H. influenzae* type b vaccine efforts, such cooperation should be the appropriate goals of academia, government, and industry in the current development and clinical testing of pneumococcal vaccines in more diverse populations.

That the avidities and functional activities of capsule-specific IgG were correlated in this study is instructive, and that each was significantly lower in this specific elderly population

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is intriguing. As noted by the authors, the elderly subjects studied were of advanced age (predominantly >80 years old) and involved in an epidemic of viral and bacterial pneumonia. The elderly subjects were institutionalized and predominantly (85%) female, characteristics which have each been associated with decreased antibody responses to pneumococcal vaccination [37–39]. That other studies have shown more comparable levels and avidities of capsule-specific antibodies in young and healthy elderly adults [40, 41] raises the issue of whether underlying disease or intrinsic "immunosenescence" or both contribute to the age-related differences reported here.

In addition, immune responses among the elderly were determined in this study at a relatively short interval (2–3 weeks) following vaccination. Although specific IgG levels rise significantly at 2 weeks after pneumococcal immunization, previous reports suggest that levels of specific antibody may peak at 70–100 days postvaccination, particularly in elderly subjects [40, 42]. Whether antibody levels rise more slowly in the advanced elderly, particularly those with underlying diseases, and whether the improvement of antibody binding or affinity maturation develops more slowly in this group are relevant areas of investigation.

Although protein-conjugate vaccines have not enhanced specific antibody levels in the elderly compared with responses to pure polysaccharides [43, 44], the authors suggest that these new vaccines may enhance avidity [45], as the two parameters are independently regulated.

Overall, most investigators would agree that more effective pneumococcal vaccines are required in the elderly, particularly the advanced elderly, among whom rates of pneumococcal disease are highest. Romero-Steiner et al. have provided solid evidence for the use of one accessible tool, antibody avidity, in the evaluation of the current and newer vaccines under development. How should their findings be applied in order to determine the most appropriate analytic tool for evaluating the efficacy of pneumococcal vaccines in this population?

First, the population studied should include a broad representation of healthy and compromised elderly adults of both sexes. Optimally, samples could be obtained prospectively from a cohort efficacy trial and tested selectively on a case-control basis. Second, each of the putative indicators of vaccine "protection," e.g., antibody levels, avidity, and functional activity, should then be correlated with clinical outcomes. Such laboratory measures could then be credibly accepted as surrogate indicators of protection, on the basis of solid clinical and epidemiological evidence. Romero-Steiner et al. have advanced us on this productive path to efficiently and cost-effectively evaluating the new generation of pneumococcal vaccines in the expanding populations at risk.

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References

1. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. Clin Microbiol Rev **1991**;4:359–95.
2. Newman SL, Vogler LB, Feigin RD, Johnston RB Jr. Recurrent septicemia associated with congenital deficiency of C2 and partial deficiency of factor B and the alternate complement pathway. N Engl J Med **1979**; 299:290–2.
3. Winkelstein JA. Complement and the host's defense against the pneumococcus. Crit Rev Microbiol **1984**;11:187–208.
4. O'Neal BJ, McDonald JC. The risk of sepsis in the asplenic adult. Ann Surg **1981**;194:775–8.
5. Selby C, Hart S, Ispahani P, Toghill PJ. Bacteremia in adults after splenectomy or splenic irradiation. Q J Med **1987**;63:523–30.
6. Phan H, Yee A, Zuniga R, Salmon J, Musher D. FcγRIIA allelic polymorphism as a determinant of susceptibility to bacteremic pneumococcal infection [abstract]. Clin Infect Dis **1998**;27:921.
7. Rodriguez ME, van der Pol W-L, Sanders LAM, van de Winkel JGJ. Crucial role of FcγRIIa (CD32) in assessment of functional anti–*Streptococcus pneumoniae* antibody activity in human sera. J Infect Dis **1999**;179:423–33.
8. Ambrosino DM, Schiffman G, Gotschlich EC, et al. Correlation between G2M(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. J Clin Invest **1985**;75:1935–42.
9. Rosen FS, Janeway CA. The gamma globulines. III: the antibody deficiency syndromes. N Engl J Med **1966**;275:709–15.
10. Siber GR, Schur PH, Aisenberg AC, et al. Correlation between serum IgG2 concentrations and the antibody response to bacterial polysaccharide antigens. N Engl J Med **1980**;303:178–82.
11. Wong WY, Powars DR, Chan L, Hiti A, Johnson C, Overturf G. Polysaccharide encapsulated bacterial infection in sickle cell anemia: a thirty year epidemiologic experience. Am J Hematol **1991**;39:176–82.
12. Zarkowsky HS, Gallagher D, Gill FM, et al. Bacteremia in sickle hemoglobinopathies. J Pediatr **1986**;109:579–85.
13. Janoff EN, Rubins JB. Invasive pneumococcal disease in the immunocompromised host. Microbial Drug Resist **1997**;3:215–32.
14. Smit P, Oberholzer D, Hayden-Smith K, Koornhof HJ, Hilleman MR. Protective efficacy of pneumococcal polysaccharide vaccines. JAMA **1977**;238:2613–6.
15. Austrian R, Douglas RM, Schiffman G, et al. Prevention of pneumococcal pneumonia by vaccination. Trans Assoc Am Physicians **1976**; 89:184–94.
16. MacLeod CM. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J Exp Med **1945**;82:445–65.
17. Farr BM, Johnston L, Cobb DK, et al. Preventing pneumococcal bacteremia in patients at risk. Arch Intern Med **1995**;155:2336–40.
18. Fine MJ, Smith MA, Carson CA, et al. Efficacy of pneumococcal vaccine in adults. Arch Intern Med **1994**;154:2666–77.
19. Shapiro ED, Berg AT, Austrian R, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N Engl J Med **1991**; 325:1453–60.
20. Simberkoff MS, Cross AP, Al-Ibrahim M, et al. Efficacy of pneumococcal vaccine in high-risk patients: results of a Veterans Administration Cooperative Study. N Engl J Med **1986**;315:1318–27.
21. Örtqvist Å, Hedlund J, Burman B-Å, et al. Randomised trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. Lancet **1998**;351: 399–403.
22. Quataert SA, Kirch CS, Quackenbush WLJ, et al. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. Clin Diagn Lab Immunol **1995**;2:590–7.
23. Musher DM, Johnson B Jr, Watson DA. Quantitative relationship between anticapsular antibody measured by enzyme-linked immunosorbent as-

say or radioimmunoassay and protection of mice against challenge with *Streptococcus pneumoniae* serotype 4. Infect Immun 1990;58:3871–6.

24. Romero-Steiner S, Libutti D, Pais LB, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin Diagn Lab Immunol 1997;4:415–22.
25. Romero-Steiner S, Musher DM, Cetron MS, et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. Clin Infect Dis 1999;29:281–8.
26. Karush F. The affinity of antibody: range, variability, and role of multivalence. In: Comprehensive immunology. New York and London: Plenum Medical Book, 1985:85–116.
27. Steward MW, Steensgard J. Antibody affinity: thermodynamic aspects and biological significance. Boca Raton, Florida: CRC Press, 1983.
28. Hetherington SV, Lepow ML. Correlation between antibody affinity and serum bactericidal activity in infants. J Infect Dis 1992;165:753–6.
29. Schlesinger Y, Granoff DM, The Vaccine Study Group. Avidity and bactericidal activity of antibody elicited by different *Haemophilus influenzae* type b conjugate vaccines. JAMA 1992;267:1489–94.
30. Granoff DM, Maslanka SE, Carlone GM, et al. A modified enzyme-linked immunosorbent assay for measurement of antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. Clin Diagn Lab Immunol 1998;5:479–85.
31. Pullen GR, Fitzgerald MG, Hosking CS. Antibody avidity determination by ELISA using thiocyanate elution. J Immunol Methods 1986;86:83–7.
32. Hetherington SV. The intrinsic affinity constant (K) of anticapsular antibody to oligosaccharides of *Haemophilus influenzae* type b. J Immunol 1988;140:3966–70.
33. Hetherington S. Solid phase disruption of fluid phase equilibrium in affinity assays with ELISA. J Immunol Methods 1990;131:195–202.
34. Friguet B, Chaffotte AF, Djavadi-Ohaniance L, Goldberg ME. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J Immunol Methods 1985;77:305–19.
35. Malmborg A-C, Borrebaeck CAK. BIAcore as a tool in antibody engineering. J Immunol Methods 1995;183:7–13.
36. Rath S, Stanley CM, Steward MW. An inhibition enzyme immunoassay for estimating relative antibody affinity and affinity heterogeneity. J Immunol Methods 1988;106:245–9.
37. Roghmann KJ, Tabloski PA, Bentley DW, Schiffman G. Immune response of elderly adults to pneumococcus: variation by age, sex, and functional impairment. J Gerontol 1987;42:265–70.
38. Sankilampi U, Honkanen PO, Bloigu A, Herva E, Leinonen M. Antibody response to pneumococcal capsular polysaccharide vaccine in the elderly. J Infect Dis 1996;173:387–93.
39. Sankilampi U, Isoaho R, Bloigu A, Kivela SL, Leinonen M. Effect of age, sex and smoking habits on pneumococcal antibodies in an elderly population. Int J Epidemiol 1997;26:420–7.
40. Rubins JB, Puri AKG, Loch J, et al. Magnitude, duration, quality and function of pneumococcal vaccine responses in elderly adults. J Infect Dis 1998;178:431–40.
41. Konradsen HB. Quantity and avidity of pneumococcal antibodies before and up to five years after pneumococcal vaccination of elderly persons. Clin Infect Dis 1995;21:616–20.
42. Musher DM, Groover JE, Rowland JM, et al. Antibody to capsular polysaccharides of *Streptococcus pneumoniae* prevalence, persistence, and response to revaccination. Clin Infect Dis 1993;17:66–73.
43. Shelly MA, Jacoby H, Riley GJ, Graves BT, Pichichero M, Treanor JJ. Comparison of pneumococcal polysaccharide and CRM_{197}-conjugated pneumococcal oligosaccharide vaccines in young and elderly adults. Infect Immun 1997;65:242–7.
44. Powers DC, Anderson EL, Lottenbach K, Mink CM. Reactogenicity and immunogenicity of a protein-conjugated pneumococcal oligosaccharide vaccine in older adults. J Infect Dis 1996;173:1014–8.
45. Antilla M, Eskola J, Åhman H, Käyhty H. Avidity of IgG for *Streptococcus pneumoniae* type 6b and 23F polysaccharides in infants primed with pneumococcal conjugates and boosted with polysaccharide or conjugate vaccines. J Infect Dis 1998;177:1614–21.